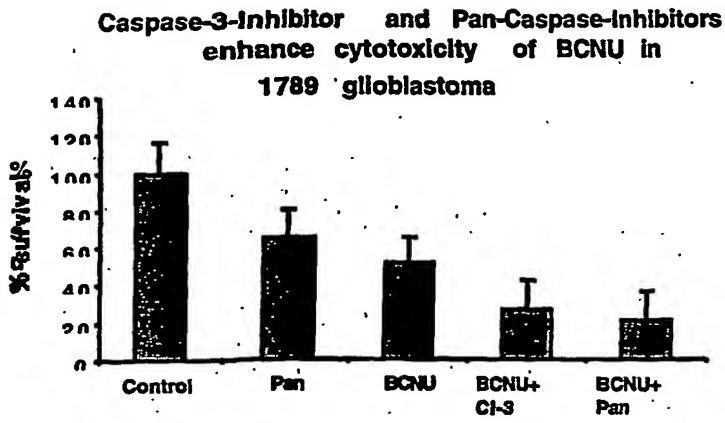


Figure 1. Exposure of the 1789 glioblastoma cell line to a pan-caspase inhibitor causes a reduction in cell number equivalent to the effects of exposure to BCNU. Combined exposure to BCNU and the pan-caspase inhibitor significantly increased the amount of cell death over that caused by exposure to BCNU alone. A similar enhancement of BCNU-induced killing was caused by co-exposure to BCNU and an inhibitor of caspase 3.

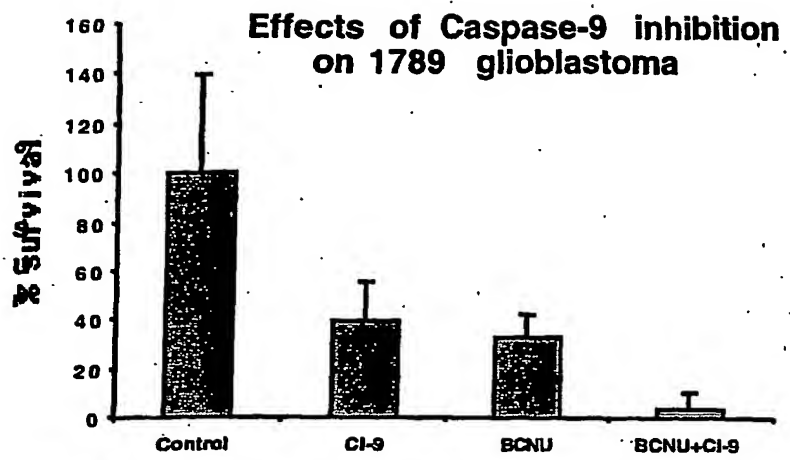


Figure 2. Exposure of the 1789 glioblastoma cell line to an inhibitor of caspase-9 causes a reduction in cell number equivalent to the effects of exposure to BCNU. Combined exposure to BCNU and an inhibitor of caspase-9 significantly increased the amount of cell death over that caused by exposure to BCNU alone.

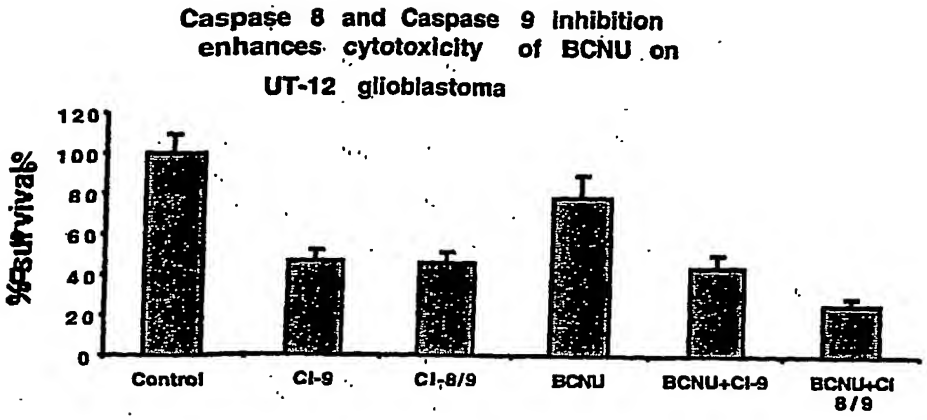


Figure 3. Exposure of the UT-12 glioblastoma cell line to an inhibitor of caspase-9 causes a reduction in cell number even greater than the effects of exposure to BCNU. Similar reductions are caused by exposure to a combination of caspase 8 and caspase 9 inhibitors. Combined exposure to BCNU and inhibitors of caspase-8 and caspase-9 applied together with BCNU was associated with significantly increased cell death over that caused by exposure to BCNU alone. In addition, the combination of BCNU and inhibitors of caspase 8 and 8 caused a significantly greater killing of cancer cells than did application of the caspase inhibitors by themselves or by the application of BCNU by itself.

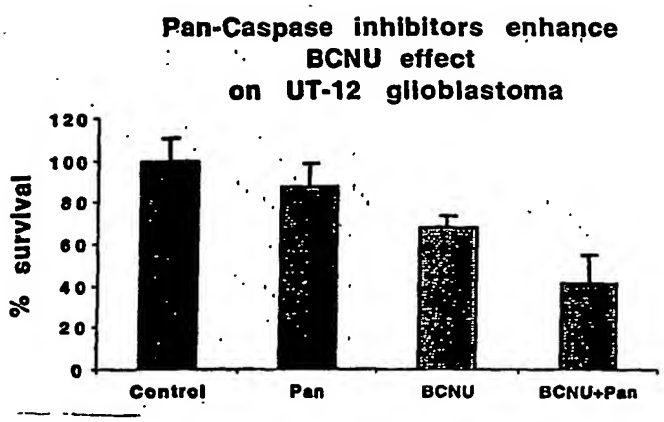


Figure 4. Combined exposure of the UT-12 glioblastoma cell line to BCNU and a pan-caspase inhibitor significantly increased the amount of cell death over that caused by exposure to BCNU alone.

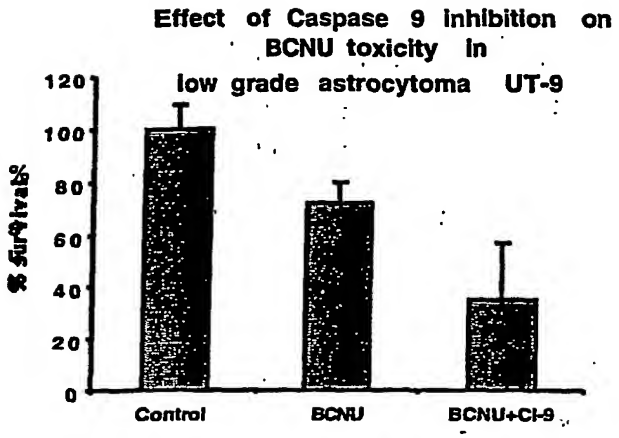


Figure 5. Exposure of the UT-9 astrocytoma cell line (derived from a low grade astrocytoma, WHO grade II) to BCNU (at equivalent doses used for the glioblastoma cell lines 1789 and UT-12) causes only a minor reduction in cell number. In contrast, when BCNU is added together with an inhibitor of caspase-9 the number of cells killed is significantly increased. These experiments that caspase inhibitor activation may also be able to overcome chemoresistance.

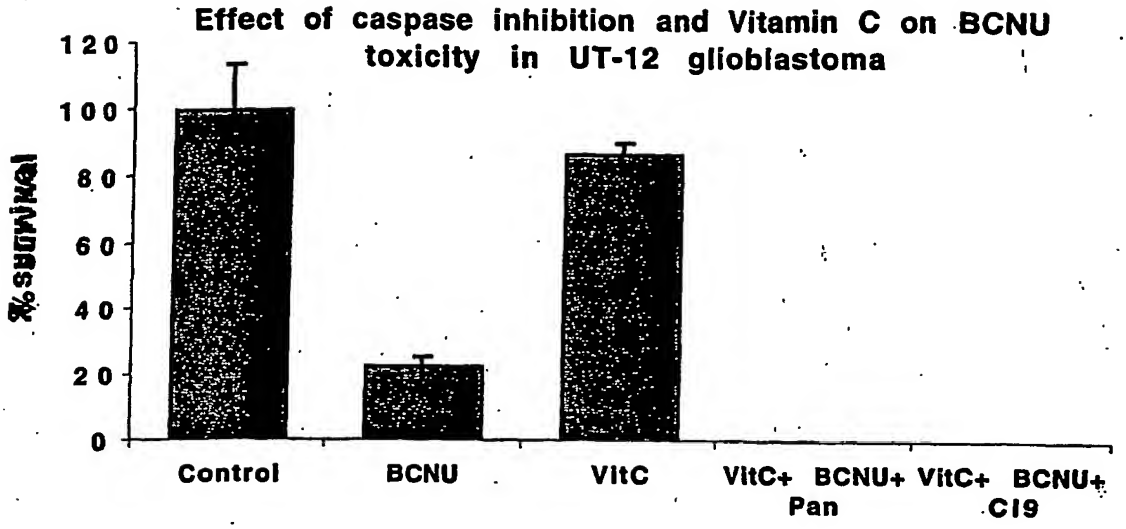


Figure 6. These experiments demonstrate that the anti-oxidant Vitamin C fails to rescue tumor cells from death induced by exposure to BCNU and caspase inhibitors. In comparison with Figure 4, one sees that the introduction of the anti-oxidant makes killing of cancer cells even more effective.

Effect of caspase inhibitors on normal brain cells:

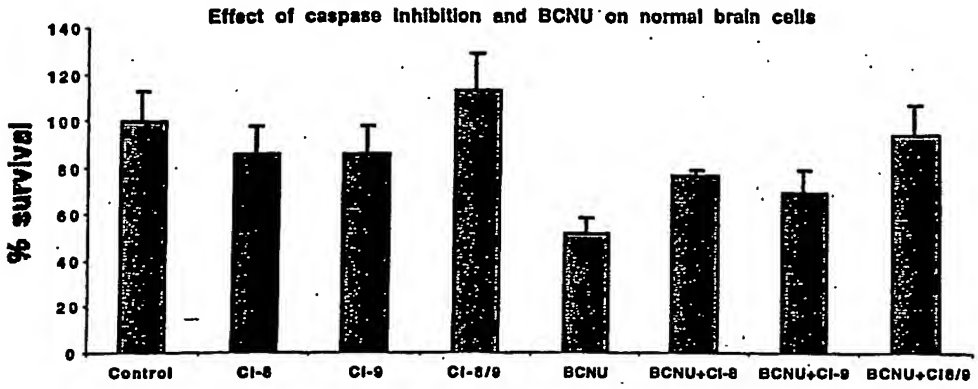


Figure 7. In contrast to the effects of caspase inhibitors in enhancing the killing of tumor cells (as shown in Fig. 1-5), these same inhibitors do not have such effects on normal human brain precursor cells. This example shows treatment of human glia restricted precursor cells (GRP) with BCNU. Caspase 8 and 9 inhibitors do not enhance the cytotoxic activity of BCNU nor do they compromise the viability of human GRP cells when applied by themselves.

Figure 8. Caspase inhibitors do not enhance the cytotoxic effects of BCNU on normal astrocytes. Cells were plated (1000cells/well) on 24-well-coverslips and exposed to BCNU (40μg/ml for 1 h) alone or to BCNU in combination with caspase inhibitors (CI-8 and CI-9). Cells were exposed to caspase inhibitors for 24hrs at a concentration of 20μM each. After a 48hr recovery period, cells were MTT/DAPI-stained to determine viability. Error bars represent s.e.m.

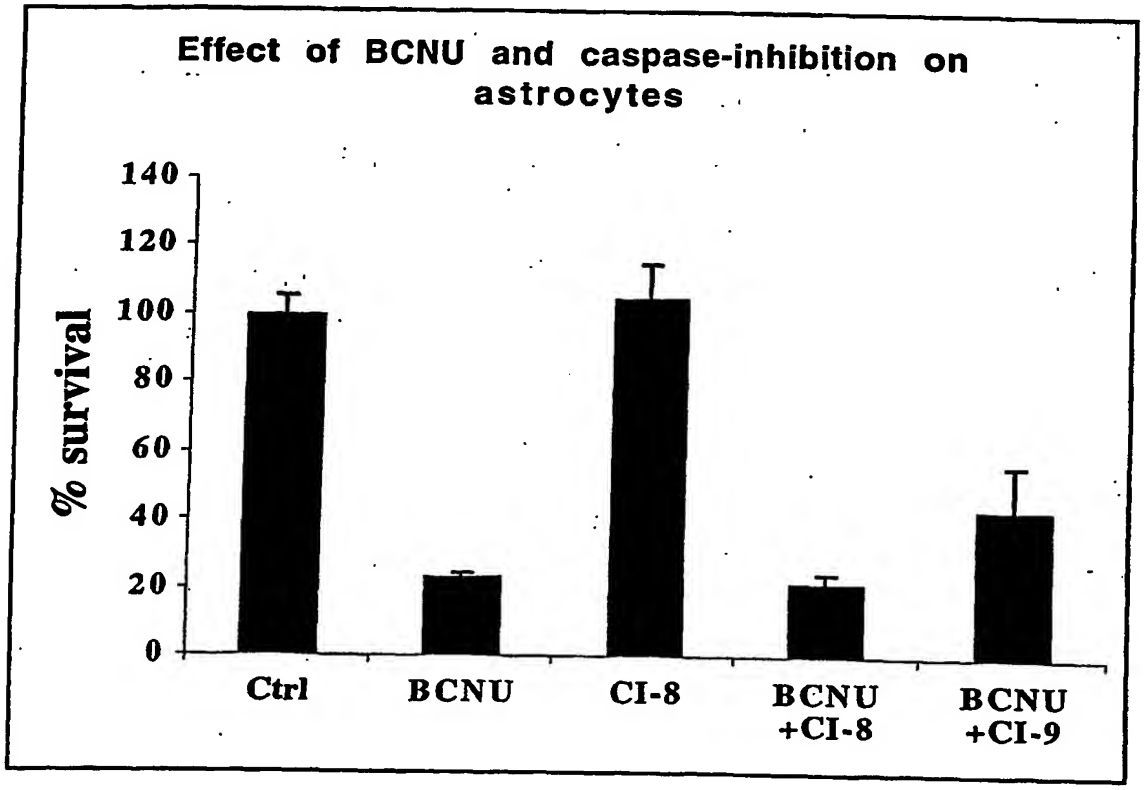
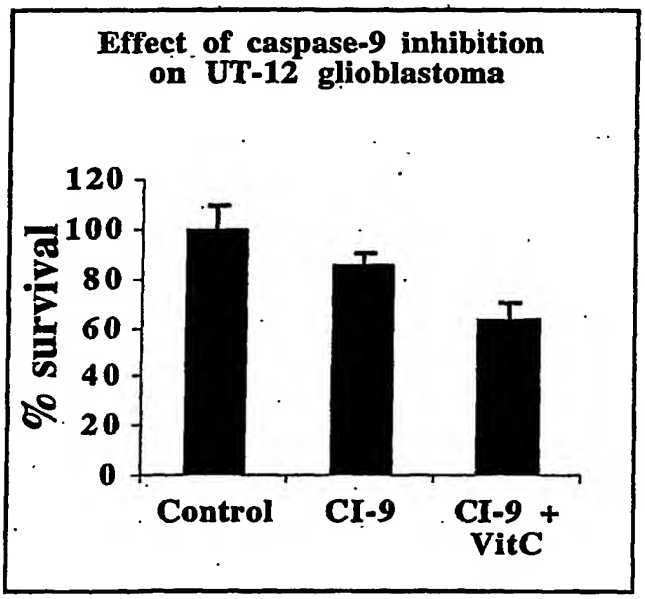


Figure 9. Caspase 9 inhibitor and Vitamin C decrease survival of UT-12 glioblastoma cells.
Cells were plated (1000cells/well) on 24-well-coverslips and exposed to caspase 9 inhibitor (20μM) ± Vitamin C (20 μg/ml) for 24 hrs. After a 48hrs recovery period, cells were MTT/DAPI-stained to determine viability. Error bars represent s.e.m.



Caspase inhibitors do not rescue cisplatin-induced toxicity on SW480 colon cancer cells.

The figure shows DAPI-staining of SW480 cells after 24hrs treatment with caspase-inhibitors ± cisplatin, thus revealing the cellular nuclei.

A. Control. B. Pan-Inhibitor. C. Caspase-3 inhibitor. D. Cisplatin. E. Cisplatin+Pan-Inhibitor. F. Cisplatin+caspase-3 inhibitor. The caspase inhibitors and cisplatin were added at a concentration of 20μM.

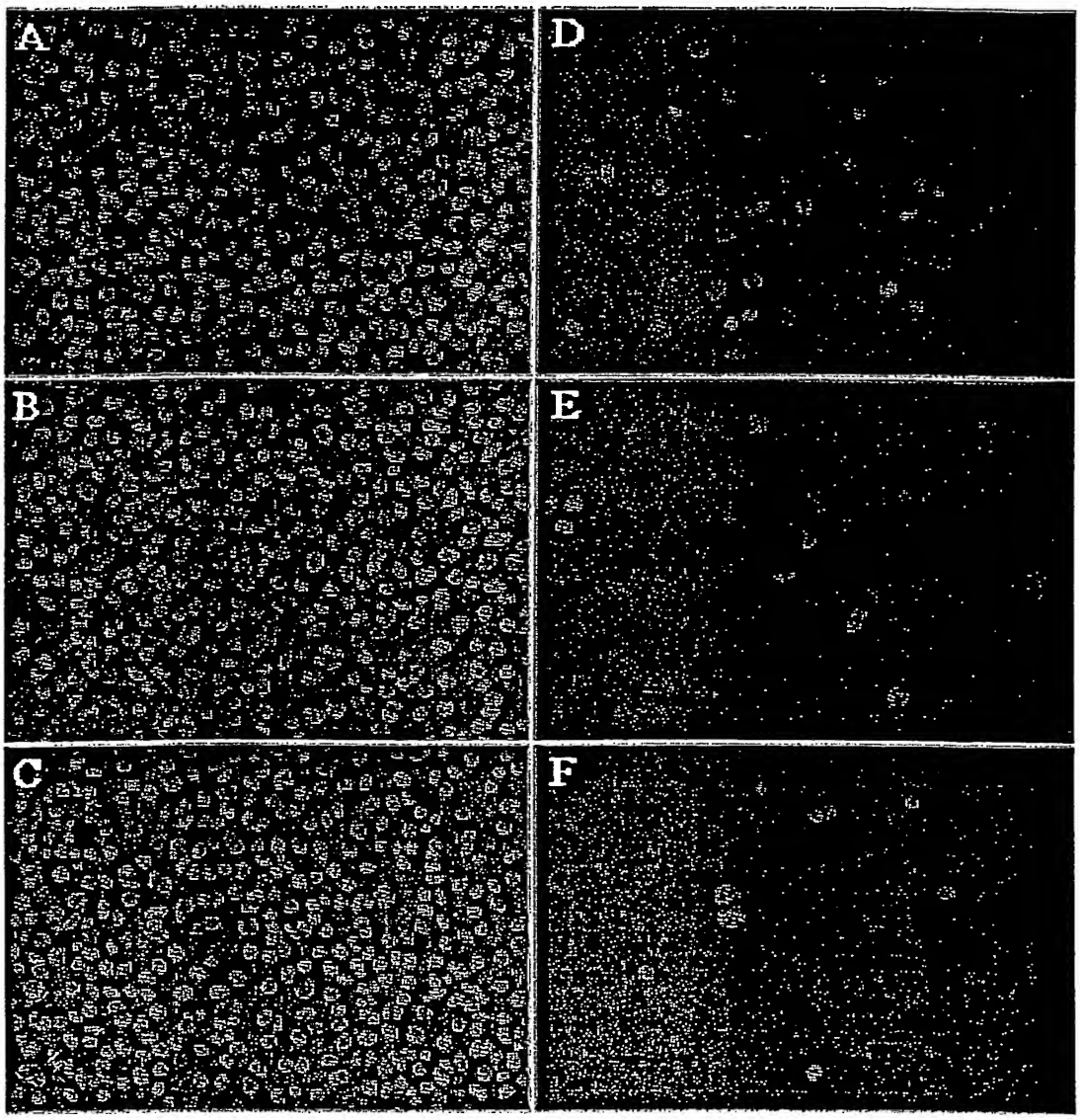


Figure 10